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ER-mitochondria interactions: both strength and weakness within cancer cells

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Abstract

ER-mitochondria contact sites represent hubs for signaling that control mitochondrial biology related to several aspects of cellular survival, metabolism, cell death sensitivity and metastasis, which all contribute to tumorigenesis. Altered ER-mitochondria contacts can deregulate Ca^{2+} homeostasis, phospholipid metabolism, mitochondrial morphology and dynamics. MAM represent both a hot spot in cancer onset and progression and an Achilles' heel of cancer cells that can be exploited for therapeutic perspectives. Over the past years, an increasing number of cancer-related proteins, including oncogenes and tumor suppressors, have been localized in MAM and exert their pro- or antiapoptotic functions through the regulation of Ca^{2+} transfer and signaling between the two organelles. In this review, we highlight the central role of ER-mitochondria contact sites in tumorigenesis and focus on chemotherapeutic drugs or potential targets that act on MAM properties for new therapeutic approaches in cancer.

Keywords: MAM; mitochondria; calcium signaling; apoptosis; cancer; therapeutic targets.

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Abbreviations

ACAT1/SOAT1, acyl-CoA:cholesterol acyltransferase 1/Sterol O-acyl transferase 1; ACC, adrenocortical carcinoma; AMBRA1, autophagy/Beclin 1 regulator 1; ACSL4/FACL4, Acyl-CoA Synthetase Long-Chain Family Member 4; ATAD3A, ATPase family AAA domain-containing protein 3A, BH domain, Bcl-2-homology domain; APEX, ascorbate peroxidase; ATP, adenosine triphosphate; BIRD-2, Bcl-2/IP3R disruptor-2; ATG, Autophagy related gene ; BAP1, BRCA1-associated protein 1; Bcl (B-cell lymphoma)-2; Ca^{2+}, ionized calcium; CAV1, caveolin-1; CL, cardiolipin; CTA, cancer-testis antigen; DRP1, dynamin-1-like protein; ER, endoplasmic reticulum; sER, smooth ER or rER, rough ER; EMR, emerin; FATE1, Fetal and Testis Expressed Transcript 1; FBXL2, F-box/LRR-repeat protein 2; FFA, free fatty acids; Fis1, mitochondrial fission protein 1; FRET, Fluorescence Resonance Energy Transfer; Grp75, glucose regulated protein 75; GRP78,/BiP glucose regulated protein 78/binding immunoglobulin protein; HCC, hepatocellular carcinoma; HCQ, hydroxychloroquine; Hk2, hexokinase 2; IMM, inner mitochondrial membrane; IP3, inositol trisphosphate; IP3R, inositol 1, 4, 5-trisphosphate receptor; IRBIT, IP3R binding protein released with inositol 1,4,5-trisphosphate; MAM, mitochondria associated membranes; MCU, mitochondrial uniporter; MFF, mitochondrial fission factor; MFN1/2, mitofusin 1/2; mTORC2, mammalian target of rapamycin 2; Miro1/2, Mitochondrial Rho GTPase; OMM, outer mitochondrial membrane; OPA1, optic atrophy type 1; ORP, oxysterol-binding protein (OSBP)-related proteins; PA, phosphatidic acid; PACS-2, phosphofurin acidic cluster sorting protein 2; PKA, protein kinase A; PML, promyelocytic leukemia; PP2A, protein phosphatase 2 A; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG, phosphatidylglycerol; PI3K, phosphoinositide 3-kinase; PS, phosphatidylserine; PSD1, Phosphatidylserine Decarboxylase 1; PSS1/2, phosphatidylserine synthase 1 and 2; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PTP, permeability transition pore; PTPIP51, proteins like protein-tyrosine phosphatase-interacting protein 51; RyRs, Ryanodine receptors; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SIGR1, sigma R1 receptor; StAR, steroidogenic acute regulatory protein; TCA, tricarboxylic acid; TG, thapsigargin; TMX1, thioredoxin-related transmembrane protein 1; TpM, trichoplein/mitostatin; TSPO, translocator protein; UPR, unfolded protein response; VAPB, vesicle-associated membrane protein-associated protein B; VDAC1, voltage-dependent anion channel 1.

1. Introduction

The interconnection between mitochondria and endoplasmic reticulum was the first interorganelle contact site discovered [1]. ER-mitochondrial contacts can be physically isolated as mitochondria-associated ER membranes (MAM) that establish a specific structure with distinct signaling functions. This microdomain works as a platform for transfer of calcium (Ca^{2+}) from the ER to mitochondria, but also exerts important functions in lipid synthesis and metabolism.

Growing interest in the past several decades revealed the mutilfaceted role of the ER -mitochondria contact sites in different cellular processes. MAM represent hubs for signaling and mechanisms that control cellular homeostasis and mitochondrial biology related to several aspects of cellular survival, metabolism, sensitivity to cell death and metastasis, all cancer hallmarks.

In this review, we will highlight the role of the ER–mitochondria interface and of its multiple physiological functions in mitochondrial Ca^{2+} signaling, metabolism and dynamics. Consistent with the pivotal role of MAM in fundamental cellular processes, we will discuss how alterations in ER-mitochondria communication impact many cancer hallmarks and play a critical role in oncogenesis.

2. MAM: structure and composition

In the 1950s, early indications of connections between ER and mitochondria have been described [1]. The distance between the ER and the OMM was originally estimated to be approximatively 100 nm. Subsequent studies using electron tomography and expression of synthetic linkers found that the ER and mitochondria are juxtaposed within a shorter distance (10 nm across at the smooth ER and 25 nm at the rough ER) [see 2 for a recent review]. Indeed, it has been recently proposed that the smooth ER (sER) is apposed to mitochondria at a distance varying from 10 to 50 nm, whereas the rough ER (rER) localizes at a distance of 50-80 nm from the mitochondrial outer membrane [3] (Figure 1). The number, length and thickness of contacts between ER and mitochondria appear as important parameters to define the biological functions of the MAM. While sER forms ER-mitochondria contacts where local phospholipid and Ca^{2+} exchange takes place, the role and dynamics of rER-mitochondria contacts are less well defined to date [4].

Many approaches have been used to elucidate the structure of ER-mitochondria contacts. Considering the short distance between cellular organelles and nanometer

resolution, electron microscopy (EM) is the preeminent methodology for direct visualization of interaction. Furthermore, tethering between the two organelles was also analyzed by electron tomography. Fluorescent proteins (FP) selectively targeted to the mitochondria and ER have been used to visualize contact sites in living cells for years, although the limited resolution in the distance range below 300 nm or alterations in organelle morphology could complicate the interpretation. To overcome these limitations, different fluorescent protein-based sensors of proximity were developed using a dimerization-dependent FP, *in situ* proximity ligation assay or rapamycin-inducible linkers tagged with a FRET-based probe [2,5]. More recently a split-GFP based contact site sensor (SPLICS) was described to monitor narrow (8-10 nm) and wide (40-50 nm) ER-mitochondria interactions by confocal microscopy in human cells and *in vivo* in zebrafish neurons [6].

In the early 1990s, the group of J. Vance isolated a subcellular fraction of the ER in close contact with the outer mitochondrial membrane (OMM) named MAM. The technique of biochemical isolation of MAM from different mammalian tissues and cultured cells was optimized and has allowed to make great progress in the MAM field over the past years [7,8]. Originally, MAM were considered as an important platform for lipid synthesis, trafficking and metabolism as demonstrated by the enrichment of several proteins in this fraction, including acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1/SOAT1) [7] that is involved in cholesterol ester formation (Figure 1) [9]. An important function of MAM in Ca^{2+} transfer and the existence of Ca^{2+} nanodomains was elucidated by the group of T. Pozzan [10]. Characterization of ER-mitochondria contact sites allowed to make significant progress in the definition of the biochemical composition of MAM and gain insight into the increasing number of their functions. Some studies used mass spectrometry-based proteomics for characterization of the MAM fraction purified by gradient centrifugation in different cellular models [11,12]. More recently, two groups used biotinylation proximity labeling by organelle-targeted ascorbate peroxydase (APEX), that labels all proteins within a few nanometer range [13,14], to characterize MAM-associated proteins. However, only two proteins, TMX1 (thioredoxin-related transmembrane protein) and calnexin, appear as commonly associated with the MAM fraction [15]. Even if caution must be taken in the interpretation of these proteomic analyses and in the consideration that in this dynamic environment not all proteins localized in the MAM

will be captured, we expect that further progress will lead to a better characterization of the cell-specific MAM proteome.

Many studies revealed the multifaceted role of MAM proteins involved in multiple functions such as Ca^{2+} signaling and transfer between ER and mitochondria, mitochondrial dynamics, autophagy, ER stress, apoptosis. Thus, ER–mitochondria contacts represent a dynamic compartment that controls different aspects of the cellular metabolism and is involved in the transmission of signals from physiological and pathological pathways harnessed by cancer cells (Table1; Figure 1). Altered ER-mitochondria contacts can deregulate Ca^{2+} homeostasis, phospholipid metabolism, mitochondrial functions and dynamics. MAM represent an important hot spot in cancer onset and progression but also constitute the Achilles' heel of cancer cells to be exploited for therapeutic perspectives.

3. MAM as a control hub of mitochondria homeostasis and metabolism

3.1. MAM and regulation of Ca^{2+} signaling

Through their intimate interaction, ER and mitochondria play a pivotal role in various aspects of cell metabolism and cell fate determination by regulating cellular Ca^{2+} signaling. Ca^{2+} is a crucial intracellular second messenger required for functional mitochondrial metabolism, bioenergetics and cell survival. The importance of MAM in the regulation of Ca^{2+} homeostasis has been strongly established [16].

The accumulation of Ca^{2+} into mitochondria mainly depends on the ER, which represents the main intracellular Ca^{2+} storage organelle, which is controlled by ER pumps and channels. Two important ER-resident proteins are involved in the transfer of Ca^{2+} and are found enriched in ER-mitochondria contact sites: the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) and inositol 1,4,5-trisphosphate receptors (IP3R) (Figure 1). The SERCA pump is localized in the ER membrane and regulates ER Ca^{2+} levels by actively pumping Ca^{2+} into the ER from cytosol creating a Ca^{2+} gradient between cytosol and the ER (from 0.1 μM to 300-1000 μM, respectively). Its SERCA2b isoform displays the highest Ca^{2+} affinity and is found enriched in MAM. Calnexin and TMX1, that are targeted to MAM in a palmitoylation-dependent manner, interact directly with and regulate SERCA2b activity [17]. Calnexin palmitoylation induces a switch of its function from quality control of protein folding to ER-Ca^{2+} signaling by enhancing SERCA2b activity. This interaction is counteracted by TMX1, that inhibits SERCA2b and promotes Ca^{2+} influx to

mitochondria. The IP3Rs and the ryanodine receptors (RyRs; mostly expressed in the sarcoplasmic reticulum) are the major Ca^{2+} release channels from the ER. IP3R isoform 3 (IP3R3) is enriched in the MAM fraction. IP3R3 is involved in Ca^{2+} transfer from ER to mitochondria and interacts physically with the mitochondrial voltage-dependent anion channel 1 (VDAC1), located in the OMM. The molecular chaperone glucose related regulated protein 75 (GRP75) acts as a bridge between IP3R3 and VDAC1 and is critical for transfer of Ca^{2+} to mitochondria. Ca^{2+} crosses the outer mitochondrial membrane through VDAC, which mediates transfer of different ions and molecules into the intermembrane space (IMS). Contrarily to the OMM, the inner mitochondrial membrane (IMM) is not Ca^{2+} permeable. Ca^{2+} is transferred to the mitochondrial matrix through the mitochondrial calcium uniporter (MCU) whose molecular identity was discovered by two different groups [18,19]. The presence of high Ca^{2+} microdomains at the interface between ER and mitochondria associated with an optimal distance between these organelles are needed for efficient Ca^{2+} delivery to the low Ca^{2+} affinity MCU [10,20,21].

These findings opened new perspectives in the mitochondria Ca^{2+} signaling field through the molecular characterization of the MCU complex, that is a multi-protein complex formed by MCU, MCUb, EMRE, MICU1 and 2, MCUR1 and SLC25A3 [see 22 for review]. The functional properties of each partner assure a tight control of Ca^{2+} concentration in the mitochondrial matrix that is important in regulating cell survival, death and metabolism. For example, Ca^{2+} increases the activity of several rate-limiting enzymes of the tricarboxylic acid cycle (TCA), including pyruvate, isocitrate, and α-ketoglutarate dehydrogenases. Recently, a link between Ca^{2+} transfer from the ER to the mitochondria and the existence of H_2O_2 nanodomains has been described [23]. ER-mitochondria Ca^{2+} transfer stimulates H_2O_2 mobilization from the mitochondrial cristae to MAM and forms specific H_2O_2 nanodomains that, in turn, sensitize ER Ca^{2+} release to stimulate Ca^{2+} oscillations and mitochondrial bioenergetics [23].

ER–mitochondria contacts exert an important role in ER stress and the unfolded protein response (UPR) involving a variety of ER chaperones localized in MAM such as the chaperone glucose related regulated protein 78 (GRP78/BiP), calnexin, calreticulin, and the Sigma 1 receptor (Sig1R). The ER protein Sig1R and the chaperone protein GRP78/BiP form a Ca^{2+} sensitive complex and prolong Ca^{2+} signaling from the ER to mitochondria by stabilizing IP3R3 [24]. The crosstalk

between ER and mitochondria regulating stress responses and UPR will not be discussed here but is the topic of an interesting recent review [17].

An important protein in ER-mitochondria contacts is Mitofusin-2 (MFN2), which belongs to the GTPase protein family and regulates mitochondrial fusion. MFN2 is enriched in MAM and controls the stability of ER-mitochondria interaction, Ca^{2+} and lipid transfer. Loss of MFN2 has been reported to reduce ER-mitochondria contact sites and to impair mitochondrial Ca^{2+} uptake [25]. However, Filadi and colleagues rekindled debate by proposing an opposite model and so the exact function of MFN2 in ER-mitochondria interaction still remains a matter of intense discussion [25,26].

Altogether, MAM represent an important molecular platform of a wide range of signaling pathways that impact several mitochondrial functions and cellular homeostasis.

3.2. MAM as an important platform for lipid synthesis and metabolism

The ER is the major site of lipid synthesis and ER-mitochondria contact sites are considered as subdomains of the ER that represent an ideal platform for lipid trafficking between organelles [see 9 for review] (Figure 1). Among the proteins enriched in MAM involved in lipid metabolism are phosphatidylserine synthases (PSS1 and 2). Essential lipids such as phosphatidylserine (PS) and phosphatidylcholine (PC) are imported from the ER to mitochondria and enzymes necessary for their biosynthesis are located in ER, MAM and mitochondria. The rate-limiting step in phosphatidylethanolamine (PE) synthesis is the transfer of PS from ER to mitochondria via the MAM. Inhibition of phosphatidylserine decarboxylase (PSD) activity resulted in the accumulation of PS in MAM confirming their essential role in the transfer of PS between the ER and mitochondria. One of the most common proteins used as a MAM marker is long chain acyl-coA synthetase (ACSL4/FACL4), which mediates the ligation of fatty acids to coenzyme A and other cholesterol metabolites [27]. Localization of ACSL4 in MAM and its important role on mitochondrial fusion has been observed during steroidogenesis in Leydig cells [28]. MAM are highly enriched in cholesterol relative to the bulk of ER and alterations of MAM cholesterol composition affect ER-mitochondria contacts [29]. MAM also contain enzymes involved in cholesterol metabolism (*e.g.* ACAT1/SOAT1) and transport (*e.g.* steroidogenic acute regulatory protein; StAR) [7,30].

A recent study identified Caveolin-1 (CAV1), an important actor of cholesterol

intracellular transport and membrane organization, as a specific component of MAM. *Cav1* genetic ablation resulted in a reduction of the stability of ER-mitochondria contact sites and an accumulation of free cholesterol in MAM [31]. In the same study, mass spectrometry characterization of proteins in the MAM fraction of mouse liver confirmed the important role of MAM in fatty acid catabolism, cholesterol and steroid metabolism. In steroidogenic cells, cholesterol transport from the OMM to IMM involves a member of the StAR family, StARD1 also known as StAR. Many data exist on the role of MAM in the synthesis of steroid hormones. A mitochondrial protein complex (steroidogenic metabolon) composed of both OMM (TSPO and VDAC2) and the IMM proteins ATPase family AAA domain-containing protein 3A (ATAD3A) and CYP11A1 form a dynamic complex that mediates cholesterol trafficking for steroid production. A recent study revealed that the long isoform of ATAD3A is involved in Leydig cells steroidogenesis through its role in MAM formation [32]. Moreover, GRP78 appears as an important regulator of steroidogenesis by regulating StAR folding at the MAM level, that is crucial for its activity and delivery to the OMM [33].

The oxysterol-binding protein (OSBP)-related proteins (ORPs) constitute a large family of lipid-binding/transfer proteins conserved from yeast (Osh) to humans (ORP). A recent study found that ORP5 and ORP8, that are ER-anchored proteins, localize to ER–mitochondria contact sites and interact with the OMM protein tyrosine phosphatase interacting protein 51 [PTPIP51; 34]. This localization is dependent on a functional lipid transfer domain and suggest that ORP5 and ORP8 mediate PS transport at ER–mitochondria contact sites. The PTPIP51 protein promotes ER–mitochondria contact through interaction with the ER-localized vesicle-associated membrane protein-associated protein B (VAPB). This interaction is critical for mitochondria-ER contacts and exerts an important function on mitochondrial Ca^{2+} uptake [35].

3.3. MAM and regulation of mitochondrial morphology

Many of the mitochondrial functions ranging from mitochondrial respiration and metabolism to Ca^{2+} homeostasis and apoptosis are linked to elaborated mitochondrial morphology changes and dynamics in the cell. Mitochondrial dynamics are crucial in response to different types of stress, modulate fusion-fission equilibrium toward fragmentation (as during apoptosis) or elongation (as during starvation), and

also their motility [36]. The molecular mechanisms governing the balance between mitochondrial fusion and fission have been discussed in detail in a recent review [37]. Several studies revealed the important role of MAM in the control of mitochondrial shape and dynamics. Proteins regulating mitochondrial shape and fusion/fission balance, like Dynamin-related protein 1 (DRP1) and MFN2, and proteins involved in mitochondrial motility, like the mitochondrial Rho-GTPases (Miro1, 2), are enriched in MAM [25,38]. MFN2 is one of the most investigated MAM-resident proteins, whose effects on MAM structure and function have been reported in different studies but is also the matter of intense debate, as discussed previously. MFN2, located both at the OMM and the ER membranes, is able to establish homotypic and heterotypic (together with the OMM-associated MFN1) ER–mitochondria interactions. Mitochondrial fission is induced at ER-mitochondria contact sites where ER tubules physically wrap around a part of the mitochondrial network and induce local fragmentation by the recruitment of DRP1 on the OMM [39].

Additional proteins, mitochondrial fission factor (MFF) and mitochondrial fission protein 1 (FIS1), which are both located at the OMM, are involved in DRP1 recruitment to mediate mitochondrial fission, even if it is unclear whether these proteins influence fission site choice [40] (Figure 1). Proteins involved in the mitochondrial fission and fusion machinery such as DRP1, MFN1 and 2 are subjected to posttranslational modifications that regulate their expression and activity [see 41 for review]. Some among the master regulators of mitochondrial motility belong to the Miro family of proteins (Miro1 and 2). Miro1 and 2 are located at the OMM and have two EF-hand Ca^{2+} binding domains to sense high levels of Ca^{2+} to regulate mitochondrial motility [38]. These proteins have an important role in tethering mitochondria to the cytoskeleton by binding kinesin, thus enabling mitochondrial movement depending on cytosolic Ca^{2+} levels. By detaching from kinesin motors, Miro proteins induce the arrest of mitochondrial movement when Ca^{2+} cytosolic concentration is high. They also enhance the fusion state of the mitochondria at resting cytosolic Ca^{2+} concentrations but promote mitochondrial fragmentation in conditions of elevated cytosolic Ca^{2+} [38]. Miro proteins then serve as Ca^{2+}-sensitive regulators both for mitochondrial motility and fusion-fission dynamics.

Altogether, regulation of mitochondrial dynamics and motility has become an active area of research with particular importance in cancer, metabolic diseases and

neurological disorders with the emerging role of mitochondrial dynamics in cell differentiation [42,43].

4. "MAM-pathy" in tumorigenesis and cancer resistance

4.1. MAM and Ca^{2+} mediated-regulation of cell death or survival and tumorigenesis

MAM appear as an important interface in the regulation of important cellular processes such as cellular survival, apoptosis, autophagy, cell invasion, which are all involved in tumorigenesis. Several reviews discussed the emerging role of MAM in different human diseases [5, 44-45]. We will discuss here the increasing evidence of the crucial role of the MAM platform on different cellular functions harnessed by cancer cells in tumorigenic processes and drug resistance and will also refer to other excellent reviews on this topic [46-47].

As discussed before, MAM exert an important function in Ca^{2+} signaling and transfer from ER to mitochondria. Impaired Ca^{2+} transfer can induce mitochondrial matrix Ca^{2+} overload and activation of the mitochondrial permeability transition pore (PTP). PTP induction leads to the increase of IMM permeability associated with a loss of mitochondrial membrane potential, which in turn induces mitochondrial swelling, OMM rupture, release of cytochrome *c* and apoptosis.

Ca^{2+} transfer from ER to mitochondria exerts a crucial role in the regulation of cell death mediated by the proapoptotic BCL2 family members such as BAX and BAK [48]. Over the past years an increasing number of cancer-related proteins, from oncogenes to tumor suppressors, have been described to be enriched in MAM and to exert their pro- or antiapoptotic functions through the regulation of Ca^{2+} transfer and signaling between the two organelles [49] (Table1; Figure 2).

Through its localization in MAM and its central role in the control of Ca^{2+} transfer between ER and mitochondria, IP3R appears as an important target regulated by several oncogenes and tumor suppressors [49] (Figure 2). Among them, the survival Akt serine threonine kinase has been shown to interact with both ER and mitochondrial partners to regulate cell survival and to be enriched in MAM [50]. Akt is able to phosphorylate IP3Rs with preference for its isoform 3 localized in MAM and counteracting apoptosis by inhibiting IP3R-mediated Ca^{2+} efflux from ER to mitochondria [51,52]. The importance of localization in MAM in the regulation of the antiapoptotic function of Akt is reinforced by the enrichment of both its negative

regulators (PTEN and PML) and its mTORC2 activator in MAM. The PTEN tumor suppressor (Phosphatase and tensin homolog) is lost in many human cancers and is the most important negative regulator of PI3K/AKT signaling pathways. A fraction of cellular PTEN is localized at the ER and in MAM and modulates Ca^{2+} transfer from the ER to mitochondria via its phosphatase activity on IP3R3 [53]. PTEN silencing impairs ER Ca^{2+} release, lowers cytosolic and mitochondrial Ca^{2+} transients and decreases cellular sensitivity to Ca^{2+}-mediated apoptosis [53].

Recently, two pathways dysregulated in several cancer types have been shown to affect IP3R expression levels and stability in MAM by regulating IP3R proteasomal turnover and sensitivity to cell death [54,55]. The PTEN tumor suppressor competes with the F-box/LRR-repeat protein 2 (FBXL-2) ubiquitin ligase for binding to IP3R3. FBXL2 ubiquitinates IP3R3, directing it for proteasomal degradation and then producing a decline in its levels [54]. PTEN antagonizes FBXL2-mediated ubiquitination of IP3R3 and stabilizes IP3R3 in the ER. Thus, the loss of PTEN impairs pro-apoptotic mitochondrial Ca^{2+} transfer and allows cancer cells to escape apoptosis.

The other tumor suppressor BRCA1-associated protein 1 (BAP1) is a deubiquitylating enzyme that promotes ER–mitochondrial Ca^{2+} transfer by stabilizing IP3R3 in the ER. BAP1 loss of function due to inactivating mutations in the *Bap1* gene results in IP3R3 downregulation and favors malignant cell survival [55]. Moreover, the same group has shown that fibroblasts from patients carrying different germline *Bap1* mutations display metabolic rewiring towards aerobic glycolysis (also known as Warburg effect) and reduced mitochondrial respiration, creating an environment that promotes tumor growth [56]. These two interesting studies highlighted the important impact of post-translational regulation of IP3R in oncogenesis, even if the interplay between BAP1 and FBXL2 should be explored. Recently, another oncogenic transcription factor, STAT3, which is involved in different processes linked to tumorigenesis (survival, drug resistance, migration and invasion), was found enriched in ER and MAM [57]. Constitutively active STAT3 reduces ER-mitochondria Ca^{2+} transfer and interacts with IP3R3 to mediate its degradation, then inducing cell resistance to apoptosis [57]. Moreover, in breast tumors displaying constitutively active STAT3, STAT3 and IP3R3 proteins levels are inversely correlated [57].

The promyelocytic leukemia protein (PML) tumor suppressor is critical in multiple apoptotic pathways as its absence inhibits cell death induced by various apoptotic stimuli. The localization of PML in MAM is essential for the induction of apoptotic pathways through Ca^{2+} transfer to mitochondria. PML interacts with IP3R3 and forms a complex with the AKT protein kinase and the PP2A protein phosphatase to regulate IP3R3 phosphorylation and activity [50]. In the absence of PML, an increase of Akt phosphorylation at the ER due to an impaired PP2A activity leads to a hyper-phosphorylation of IP3R3, which reduces Ca^{2+} flux from ER to mitochondria rendering cells resistant to apoptotic Ca^{2+}-dependent stimuli [50] (Figure 2). Another IP3R-interacting protein, mTORC2, is associated with MAM in a growth factor-stimulated manner. mTORC2/Akt at MAM interacts with the IP3R-Grp75–VDAC complex and regulates IP3R3 phosphorylation and release of Ca^{2+} from the ER to mitochondria, then controlling apoptosis [58]. mTORC2 modulates MAM integrity through phosphorylation of phosphofurin acidic cluster sorting protein 2 (PACS2) by Akt. In addition, mTORC2 controls mitochondrial metabolism through the Akt-dependent-phosphorylation of the glycolytic enzyme hexokinase 2 (HK2) [58]. HK2 is localized in mitochondria and its phosphorylation by Akt stabilizes its binding to the MAM-resident protein VDAC1, that stimulates glycolysis and participates to the Warburg effect in cancer cells [58].

The p53 transcription factor is one of the most important tumor suppressor proteins in the cell. A recent study revealed a novel non-transcriptional role for cytosolic p53 through its localization in ER and MAM. Under basal conditions, p53 was found to localize at the ER, the MAM and the cytosol [59]. In response to stress induced by chemotherapeutic drugs like adriamycin or oxidative stress induced by H_2O_2, a fraction of p53 accumulates at the ER and the MAM and modulates Ca^{2+} homeostasis [54]. p53 directly interacts with SERCA, inducing a change of its oxidative state and promoting Ca^{2+} uptake in the ER. Modulation of SERCA activity affects sensitivity to apoptosis under stress conditions by influencing transfer of Ca^{2+} from ER to mitochondria in MAM [59].

Considering the recent characterization of the MCU complex and crucial role in mitochondrial Ca^{2+} uptake essential for many cellular functions, its role in tumorigenesis starts to emerge in several studies and has been discussed in a recent review [60]. MCU is overexpressed in several cancers such as colorectal, ovarian, prostate and breast cancer and is associated with genetic alterations as gene

amplifications. Marchi and colleagues identified the first MCU-targeting microRNA (miR-25) and showed that its overexpression reduces the expression of MCU, mitochondrial Ca^{2+} uptake and the sensitivity of cancer cells to several apoptotic stimuli [61]. A recent study established a correlation between MCU expression and tumor size in triple-negative breast cancer and showed that MCU is involved in cell migration and invasion [62]. Emerging evidence supports the role of mitochondrial Ca^{2+} dynamics in cell migration and a metastasis-promoting role for the MCU-dependent mitochondrial Ca^{2+} uptake in different tumors [62,63].

4.2. MAM and autophagy

Autophagy is a lysosome-mediated process for the recycling of cytoplasmic materials and organelles. It represents an important pathway to preserve cellular integrity and homeostasis and an alternative to supply energy in nutrient-deprived conditions. Several studies revealed the important dual role of autophagy both in pro-survival or pro-apoptotic mechanisms although its exact roles during different stages of tumorigenesis are not clear [64]. The role of autophagy as a tumor suppressor mechanism was suggested by the finding that many molecular actors involved in autophagy are frequently deleted or mutated in many types of tumor cells. For example, Beclin 1, a critical autophagy-regulating gene deleted in ~50% of human breast, ovarian and prostate cancers, is considered as a haploinsufficient tumor suppressor [65]. Beclin 1 is an essential protein involved in autophagy induction and in the formation of autophagosomes [for review see 66]. It belongs to the class III phosphatidylinositol 3 kinase complex (PI3K complex III) including the phosphatidylinositol 3-kinase catalytic subunit type 3 (PIK3C3/Vps34), Vps15 and autophagy-related protein 14 (ATG14). Beclin 1 is tightly regulated through direct interactions with proteins such as autophagy/Beclin 1 regulator 1 (AMBRA1) and Bcl-2 that induce or inhibit autophagy, respectively [66]. Beclin 1 is inhibited by Bcl-2 via its BH3 domain and this interaction can be disrupted by phosphorylation of Bcl-2 and Beclin 1 or Beclin 1 ubiquitination. In addition, the cross-talk between oncogenic kinases and autophagy proteins represents an important mechanism involved in tumorigenesis. For example, Akt inhibits autophagy in a mTOR-independent manner via the phosphorylation and inhibition of Beclin 1 autophagic activity by recruiting it to the cytoskeleton [67].

ER-mitochondria contacts appear as an important platform in autophagosome formation and play a critical role in autophagy [68] (Figure 2). ATG5 and ATG14, which are involved in the autophagic machinery process, and other key proteins involved in autophagosome formation such as Beclin 1 and Vps34, are recruited to ER–mitochondria contact sites after starvation [68]. Furthermore, MAM integrity depending on MFN2 and PACS2 is essential for autophagosome formation [68].

Some MAM resident and tethering proteins regulate autophagy [69]. Modulation of tethering by downregulation or overexpression of VAPB and PTPIP51, that decrease and increase ER-mitochondria contacts, stimulates or impairs autophagy, respectively. Moreover, these effects are mediated through their role in Ca^{2+} transfer from ER to mitochondria and importantly depend on the nature of the autophagic stimulus [69].

Interestingly, opposing studies proposed that Ca^{2+} signaling acts both as an inhibitor and as an activator of autophagy, as discussed in an excellent recent review [70]. IP3R has been proposed to be an important negative regulator of autophagy. In particular, its inhibition or depletion strongly induces autophagy independently of its Ca^{2+}- release activity [71]. IP3R is targeted directly by Beclin 1 in its N-terminal suppressor domain and enhances the formation of a complex with Bcl-2/Beclin 1. Thus, Bcl-2-mediated sequestration of Beclin 1 reduces the pool of soluble Beclin 1 protein available for autophagy induction [71]. Although these studies indicate that IP3R inhibits autophagy by sequestering Beclin 1, further studies found that IP3R promotes autophagy through IP3R/Beclin 1 complex formation [72]. During starvation-induced autophagy, increased Beclin 1 binding to IP3R appears to enhance IP3R-mediated Ca^{2+} signaling by increasing ER-Ca^{2+} stores content via upregulation of calreticulin and GRP78/BiP. Excellent reviews highlighted and critically discussed both the pro- and anti-autophagic roles of intracellular Ca^{2+} signaling and IP3R, depending on the amplitude and spatio-temporal characteristics of Ca^{2+} signaling induced by different stimuli and also on the specific state of the cell (70, 73). Different reports confirmed the complex role of Ca^{2+} transfer from ER to mitochondria and autophagy in tumor progression. Recent studies described the critical role of constitutive IP3R-Ca^{2+} release to mitochondria in the regulation of autophagy in normal and cancer cells [74,75]. Constitutive IP3R-mediated Ca^{2+} transfer from ER to mitochondria appears to be essential to support oxidative phosphorylation and mitochondrial ATP production in many cell types. In normal

cells, inhibition of the constitutive Ca^{2+} transfer to mitochondria by inhibiting IP3R impairs ATP production and leads to the activation of AMP-activated kinase (AMPK) and induction of a pro-survival autophagy mechanism [74]. In cancer cells, constitutive IP3R-mediated Ca^{2+} transfer to mitochondria appears essential for cancer cell survival by delivering an adequate Ca^{2+} supply to the mitochondrial matrix to sustain its metabolism. However, considering that cancer cells proliferate despite the low energy status of the mitochondria, autophagy induction becomes insufficient to maintain cell survival due to deficiency of nucleotide availability, resulting in a massive necrotic cell death [75]. This study revealed an essential dependency of cancer cell viability on IP3R-Ca^{2+} ER-mitochondrial transfer, considering that continuous supply of metabolic intermediates is important for lipid, nucleic acid and protein synthesis for cancer cell proliferation, as reviewed [74-76].

In addition, tumor suppressors and several oncogenes localized in MAM appear to exert important functions in autophagy. Recently, the PML tumor suppressor has been reported to act as a repressor of autophagy by inhibiting autophagosome biogenesis [77]. This study reported that PML loss inhibits IP3R-mediated Ca^{2+} transfer from ER to mitochondria and stimulates pro-survival autophagy. Furthermore, PML localization in MAM is dependent on p53 and is critical for the repression of autophagy. In the absence of p53, PML localization in MAM is lost and is not able to exert its pro-apoptotic function. Then MAM localization of PML plays an important function through the regulation of Ca^{2+} transfer from ER to mitochondria both in apoptosis and autophagy [77]. This report contrasts with the study of Cardenas and colleagues that showed that autophagy induction is not sufficient for cancer cell survival and induces cell death [75,77]. Moreover, this study showed that PML absence promotes tumor progression associated with increased autophagy and drug resistance and suggested that pharmacological inhibition of autophagy could restore chemotherapy-induced apoptosis [77].

4.3. Functional alterations of ER-Mitochondria distance in cancer cells

The control of the distance between ER and mitochondria represents a critical checkpoint in the regulation of cell function and another mechanism for control of cell survival (Figure 2). Tightening the physical contacts between those organelles using synthetic linkers leads to mitochondrial Ca^{2+} overload and apoptosis. Inversely,

loosening without disrupting these ER-mitochondria contacts sites stimulates mitochondrial respiration and ATP production [21].

An example of a protein involved in regulation of ER-mitochondria distance is Trichoplein/Mitostatin (TpM). This protein binds to keratins 8 and 18, which are the major intermediate filaments in epithelial cells and is downregulated in epithelial cancers. TpM acts as a negative modulator of ER–mitochondria juxtaposition, inhibiting apoptosis by Ca^{2+}-dependent stimuli through interaction with MFN2 in MAM [78]. Another study demonstrated the role of TpM in the inhibition of cell migration, invasion and tumorigenicity of prostate cancer cells and showed that TpM is down-regulated in advanced stage human prostate cancers [79]. Thus, TpM could act as a tumor suppressor and analysis of its expression may represent a useful clinical marker for diagnosis and prognosis in this human cancer.

NogoB/Reticulon is a member of the reticulon family that regulates ER tubular structure [80]. Increased expression of NogoB induced by hypoxia in smooth muscle cells increases the distance between ER and mitochondria and decreases ER-mitochondria phospholipid and Ca^{2+} transfer as well as mitochondria-dependent apoptosis [81]. A recent study revealed that Nogo-B is overexpressed in hepatocellular carcinoma (HCC) with an oncogenic role in HCC progression [82]. NogoB expression is up-regulated in human colorectal cancer and also appears to play an important negative role in apoptotic signaling through its interaction with the master antiapoptotic c-FLIP protein in colorectal cancer cells [83].

A recent study demonstrated that another IP3R-binding protein, IRBIT [IP3R binding protein released with inositol 1,4,5-trisphosphate] regulates apoptosis by modulating ER-mitochondria Ca^{2+} transfer by two different mechanisms [84]. IRBIT interacts with the IP3 binding domain of IP3R in a phosphorylation-dependent manner. Bcl-2-like 10 (Bcl2l10) is a member of the anti-apoptotic Bcl-2 protein family and also regulates IP3R through binding to its IP3 binding domain and subsequent inhibition of IP3R-induced Ca^{2+} release. Bonneau and colleagues showed that, at the resting state, a fraction of (phosphorylated) IRBIT and Bcl2l10 interact and form a complex with IP3R in MAM to exert an additive inhibition of IP3R activity and Ca^{2+} transfer to mitochondria [84]. Considering that unphosphorylated IRBIT interacts with Bcl2l10 but not with IP3R, they showed that under apoptotic conditions IRBIT is dephosphorylated and dissociated from IP3R together with Bcl2l10. Removal of IRBIT/Bcl2l10 from MAM relieves IP3R inhibition and induces an increase of ER-

mitochondria Ca^{2+} transfer and apoptosis. Furthermore, IRBIT appears to participate to the stabilization and length of ER-mitochondria contact sites although further characterization of the molecular mechanism is necessary.

A more recent example illustrating the important function of mitochondria-ER interaction in the regulation of apoptosis and tumorigenesis is represented by FATE1. FATE1 (Fetal and Adult Testis Expressed 1) encodes a cancer-testis antigen (CTA), a protein whose expression is restricted to testis within normal tissues, but is also expressed in some cancer types such as HCC and against which a humoral immune response can be detected in some patients [85]. In malignancy, CTA gene regulation is aberrant, resulting in their expression in a proportion of tumors of various types. Some CTA are regulated epigenetically whereas others are controlled primarily by tissue and subtype-specific transcription factors. Our group showed that FATE1 expression is activated in adrenocortical carcinoma cells by SF-1 overexpression. Steroidogenic Factor-1 (SF-1/NR5A1) is a tissue specific transcription factor that plays a pivotal role in adrenocortical development, steroidogenic function and tumorigenesis [86]. We showed that FATE1 expression is low in normal adrenal cortex and in benign tumors (hyperplasia and adenoma) but is expressed at higher levels in adrenocortical carcinoma. Interestingly, high FATE1 expression in the tumor is a poor prognosis indicator in ACC patients [87]. Our recent study revealed that FATE1 is localized at the interface between mitochondria and ER and is enriched in MAM. FATE1 exerts an anti-tethering function and is associated with a decrease in mitochondrial Ca^{2+} uptake. These effects are associated with increased resistance of adrenocortical cells to oxidative stress and apoptosis induced by Ca^{2+}-mediated stimuli or by mitotane treatment, a drug commonly used in the therapy of advanced ACC [87]. Remarkably, a previous siRNA screening study also identified FATE1 as one of the genes that sensitizes a panel of non-small cell lung cancer cell lines to paclitaxel toxicity [88]. Another study showed that FATE1 inhibits proapoptotic signaling in a variety of cancer cell lines by destabilizing the pro-apoptotic BIK protein [89], even if this pathway was not modulated by FATE1 in adrenocortical cell lines. Altogether these data suggest that FATE1 through its ER-mitochondria anti-tethering function has an important role in directing the aggressive phenotype of ACC and confers resistance to chemotherapeutic drugs in other cancer types.

4.4. Mitochondrial morphology and dynamics in cancer cells

Together with the appreciation of the importance of MAM-resident proteins in mitochondrial morphology and motility, an increasing number of studies have been dedicated to the deregulation of mitochondrial dynamics and motility in cancer, as discussed in different recent reviews [41, 43].

Briefly, it appears that mitochondrial shape is plastic during cell cycle progression with observation of highly fused mitochondria during G1/S to maintain sustained ATP production whereas fission increases during S/G2M to distribute equal amounts of mitochondria among daughter cells [43]. Fragmented mitochondria have been observed in many cancer cells, such as gliobastoma, lung, metastatic breast cancer, associated with high expression or activation of DRP1 and downregulation of MFN2 [90]. It has been shown that restoration of a fused phenotype by inhibiting DRP1 or overexpressing MFN2 impairs cell growth and increases apoptosis, suggesting the important role of mitochondrial dynamics in tumorigenesis. Altered expression of DRP1 and MFN1 associated with fragmented mitochondria was described in invasive breast tumor cells and lymph node metastasis [91]. These authors demonstrated a new role of mitochondrial dynamics in the mechanism of cancer cell migration and invasion by showing that induction of mitochondrial fission promotes mitochondrial trafficking to lamellipodia and increases migration while induction of mitochondrial fusion in turn inhibits migration of breast cancer cells. Moreover, two different studies demonstrated that altered mitochondrial dynamics is a key characteristic of K-Ras dependent cellular transformation mediated by DRP1 activity [92]. Oncogenic Ras or activation of MAPK signaling induces mitochondrial fragmentation by DRP1 phosphorylation on serine 616, while inhibition of DRP1 activity is sufficient to block pancreatic cancer growth in a xenograft model [92,93]. Interestingly, a recent study revealed a new role of mitochondrial dynamics in tumor cells exposed to PI3K therapy currently used in the clinic [94]. Treatment with PI3K antagonists reactivated Akt/mTOR signaling in tumor cells and induced an increase of trafficking and elongated mitochondria to the cortical cytoskeleton of tumor cells. This redistribution of energetically active mitochondria supports increased lamellipodia dynamics associated with a turnover of focal adhesion complexes and increased tumor cell migration and invasion. These data reinforce the important role of adaptative mitochondrial dynamics and reprogramming in drug resistance and invasion processes of cancer cells.

5. MAM "A contact before dying": new therapeutic strategies in cancer

In this review, we tried to highlight the central role of ER-mitochondria contact sites in different steps of tumorigenesis involving many targetable key actors that are niched in MAM. MAM are a critical hub for different functions such as Ca^{2+} signaling, lipid metabolism, mitochondrial morphology and autophagy, even if this list of cellular functions is far to be exhaustive. We would like to focus now on chemotherapeutic drugs or potential targets that act through their MAM localization and that can affect cancer properties (Figure 2).

5.1. By targeting calcium signaling

The important function of MAM on mitochondrial Ca^{2+} transfer and signaling plays a major role in the cytotoxic effects of chemotherapeutic drugs, as discussed recently [95] (Figure 2). The major actors involved in Ca^{2+} transfer between ER and mitochondria, such as VDAC, SERCA2b, IP3R, represent potential targets for pharmacological regulation and cancer chemotherapy. VDAC represents a potential therapeutic target that has been extensively reviewed recently [96]. A selective inhibitor of the SERCA pump, thapsigargin (TG), is used in research to inhibit Ca^{2+} uptake into ER and deplete ER Ca^{2+} stores. The G202 compound (Mipsagargin) was generated by coupling a prostate-specific membrane antigen (PSMA)-specific peptide to an analogue of the potent TG compound. PSMA is a type II membrane carboxypeptidase overexpressed in prostate cancer cells and most tumor endothelial cells, but not in normal vasculature or normal tissue epithelium. G202 is a non-toxic prodrug by itself that is activated specifically in tumors since it binds to PSMA and is then cleaved, liberating the peptide that exerts its toxic effect on PSMA-expressing epithelial cells and tumor vessels. G202 significantly inhibits progression in prostate, breast and bladder cancers, while presenting minimal toxicity to the host animals [97]. Mipsagargin has showed promising results in several pre-clinical studies and is currently in phase II clinical trial for prostate cancer and progressive glioblastoma.

Resveratrol is a natural phenol in several plants like grapes with pleiotropic activities as an antioxidant, anti-inflammatory compound [98]. Resveratrol and its derivative piceatannol cause cell death in cancer cells by inhibiting ATP synthase and subsequently decreasing SERCA activity. Resveratrol appears to affect ER-mitochondria contacts and enhances mitochondrial Ca^{2+} overload and, ultimately, apoptotic cell death selectively in cancer cells [99]. Resveratrol appears to be a

promising anti-cancer agent and has been used in clinical trials conducted in patients with colorectal cancers.

Adriamycin (or Doxorubicin) inhibits topoisomerase II and is a widely used chemotherapeutic drugs in various types of cancer such as breast, ovarian and lung cancers. Interestingly, adriamycin induces accumulation of p53 at the ER and MAM, associated with an increase of SERCA activity, ER Ca^{2+} levels and Ca^{2+} transfer from ER to mitochondria and apoptosis in cancer cells [59]. Another chemotherapeutic drug widely used for treatment of various cancers is cisplatin, a platinum-based drug that binds to purines in DNA but presents important side effects associated with acquired drug resistance during therapy. Some studies have shown that cisplatin increases Ca^{2+} release from the ER to cytosol and mitochondria through an increase of ER-mitochondria contact sites, inducing apoptosis in cisplatin-sensitive SKOV3 ovarian cancer cells. The antiapoptotic protein Bcl-2 inhibits cisplatin-induced ER Ca^{2+} release into mitochondria by reducing the number of ER-mitochondrial contacts in SKOV3 cells and appears as a novel marker of cisplatin resistance in ovarian cancer cells [100]. In addition to regulating cell fate via interaction with other BCL2 protein family members, Bcl-2 modulates Ca^{2+} signaling acting on ER-mitochondria interaction, as reviewed [101]. Many cancers highly express the antiapoptotic Bcl-2 proteins, that represent potential therapeutic targets in chemotherapy. Prolonged treatment of ovarian cancer cells with drugs such as ABT-737, a Bcl-2-inhibitor acting as a BH3 mimetic drug, has been proposed to alter ER-mitochondrial Ca^{2+} transfer and sensitivity to chemotherapy [102]. These BH3 mimetics function by binding into the hydrophobic groove of Bcl-2 and Bcl-XL, thereby antagonizing their capacity to inhibit apoptosis. Since Bcl-XL activity is essential for platelet differentiation and survival, non-selective BH3 mimetic Bcl-2/Bcl-XL antagonists may produce thrombocytopenia.

At the ER level, Bcl-2 regulates IP3R activity by binding to IP3R through its BH4 domain. Recently, a cell-permeable peptide compound, BIRD2 (Bcl-2/IP3R disruptor-2), that inhibits interaction between Bcl-2 and IP3R, has been shown to induce apoptosis in different cancers, including diffuse large B cell lymphoma, chronic lymphocytic leukemia and many solid tumors [103]. BIRD2 abolished Bcl-2 inhibitory effect on IP3R by targeting IP3-mediated Ca^{2+} signaling. It induced an IP3R-mediated Ca^{2+} release from ER stores that caused a marked intracellular Ca^{2+} elevation, leading to Ca^{2+}-mediated apoptosis which is in part probably mediated by

mitochondrial Ca^{2+} overload [104]. BIRD2 is a stabilized 20 amino acid peptide that corresponds to the Bcl-2-binding site on the regulatory and coupling domain of IP3R and functions as a competitive inhibitor of the Bcl-2 – IP3R interaction. Moreover, in primary lymphoma and leukemia cells BIRD2 sensitivity correlates with the level of constitutive IP3 signaling and with the expression of IP3R isoform 2, which is the isoform with the highest sensitivity to its IP3 ligand. Despite a possible differential modulation of IP3R isoforms and Bcl-2 depending on the cellular context, major efforts are being made in the development of drugs that target the dual role of anti-apoptotic Bcl-2 family members at the mitochondria and at the ER via BH3- and BH4-targeting drugs. These works have progressed in different clinical studies, as discussed in more detail in a recent interesting review [105].

In addition, the strategy based on inhibition of IP3R3 degradation using GGTi-241, a geranylgeranyl transferase inhibitor and inhibitor of FBXL2, restored apoptosis in PTEN-deregulated cancer cells. Moreover, GGTi-241 sensitized xenotransplanted tumors to photodynamic therapy and is actually in a phase I clinical trial [54].

5.2. By targeting autophagy

Defects in autophagy are linked to early tumorigenesis in a number of mouse models and represent an important mechanism involved in chemotherapy resistance in leukemia and other cancers [106]. Pharmacologic or genetic inhibition of autophagy has been shown to promote the response of tumor cells to chemotherapy, radiation therapy and immunotherapy in many cancers, as reviewed recently [64,106].

Considering the sensitizing effect of autophagy inhibition on chemotherapy-induced cell death, a number of clinical trials have been initiated by combining the autophagy inhibitor hydroxychloroquine (HCQ) with individual chemotherapeutic agents in patients with solid tumors. HCQ is a widely used antimalarial and antirheumatic drug with a well-defined toxicity profile and a known inhibitor of autophagy. Its involvement in the inhibition of autophagy is due to its impact on lysosomal acidification, followed by blocking the fusion of autophagosomes with lysosomes. However, this drug carries a significant risk of irreversible blindness from HCQ retinopathy.

Resveratrol can also induce autophagy via both mTOR-dependent and -independent mechanisms involving mitochondria Ca^{2+} signaling pathways and depending on the presence of IP3Rs and on the availability of cytosolic Ca^{2+} [107]. Altogether, these studies open new possibilities in the potential clinical application of targeting MAM

resident actors in autophagy in addition to conventional chemotherapy for treatment of many tumors.

5.3. By targeting lipid metabolism and cholesterol trafficking

Lipid composition of MAM is important for the regulation of Ca^{2+} homeostasis and ER-mitochondria contact sites and appears essential in the regulation of tumorigenesis and cancer resistance. Mitochondria have a singular lipid composition with phosphatidylglycerol, cardiolipin (CL), and phosphatidylethanolamine and low amounts of cholesterol [9].

Lipid composition alterations in mitochondrial membranes are expected to impact the mitochondrial functions, dynamics and response to apoptotic stimuli in cancer cells as already described for CL, which is considered as a mitochondria-specific phospholipid. CL microdomains play important roles in apoptosis and autophagy [9]. During apoptosis, CL is redistributed between the IMM and the OMM and CL-mediated changes in the structure and curvature of the membrane constitute a platform for recruitment of multi-protein complexes and apoptosis induction. CL is required for the structural assembly of the mitochondrial respiratory chain. Recently, the disassembly of respiratory chain complex II due to CL loss was proposed to be responsible for cell death triggered in response to mitochondrial Ca^{2+} overload [108]. Moreover, CL affects the activity and stability of many membrane protein complexes and ATP/ADP carrier proteins and regulates mitochondrial fusion and fission [109].

As discussed previously, MAM is an important platform for cholesterol metabolism. Cholesterol accumulation has been reported in several solid tumors and modulates tumor cell growth and cell survival through activation of Akt pathways [110].

STAR and STARTD3 are two essential proteins localized in MAM that regulate cholesterol import into mitochondria. In HCC, increased mitochondrial cholesterol content is associated with increased expression of STAR and its knockdown increased sensitivity to chemotherapeutic agents [111]. STARTD3 overexpression is associated with a poor prognosis for breast cancer patients and may contribute to breast cancer aggressiveness by increasing membrane cholesterol content and inducing metastases by decreasing adhesion of breast cancer cells [112].

Our recent study showed that FATE1 overexpression protects cells from apoptosis induced by mitotane, that is the current medical therapy for advanced adrenocortical tumors [87]. Mitotane induces the accumulation of cytotoxic free cholesterol,

oxysterols and fatty acids and triggers ER stress within adrenocortical carcinoma cancer cells by inhibiting MAM-localized ACAT1/SOAT1 [113]. Expression of this enzyme is correlated with response to mitotane in adrenocortical tumors. These data are particularly relevant as high FATE1 expression in the tumor is a poor prognosis indicator in ACC patients [87]. Finally, pharmacological inhibitors of the ACAT1/SOAT1 enzyme have been shown to suppress tumor growth in prostate cancer xenograft models by limiting cholesteryl ester storage and clinical trials are underway in ACC [114]. Moreover, mitotane appears to synergize with other pharmaceutical agents to induce toxicity in ACC cells. The novel GRP78/BiP inhibitor HA15 induces ER stress and can synergize with mitotane to induce cell death in human adrenocortical H295R cells, demonstrating that therapeutic interference with ER stress pathways may be useful in adrenocortical carcinoma [115]. GRP78/BiP appears to play a key role in breast and colon tumor cells, by cooperating with ATAD3A, a mitochondrial and also MAM resident protein, to stabilize WASF3, a protein that facilitates actin polymerization, thereby promoting invasion and metastasis [116].

6. Conclusions

In conclusion, MAM represent an important intracellular signaling hub, in particular for Ca^{2+} and lipid metabolism, that exerts a crucial role in the control of cell death, autophagy, mitochondrial metabolism, morphology and dynamics. These ER–mitochondrial interactions are altered during tumorigenesis to promote different cancer hallmarks such as cell survival, drug resistance and metastasis. The emerging concept that cancer cells are addicted to constitutive ER–mitochondrial Ca^{2+} transfer represents one therapeutic strategy to target tumor cells by suppressing their survival and invasion.

However, a major challenge will be to limit these effects to cancer cells and the identification of cancer-specific MAM-resident proteins is promising for the development of new approaches in cancer treatment. Targeting MAM structure, function, and dynamics will open new perspectives in the therapeutic repertoire for other human pathologies involving “MAM-pathies” such as neurodegenerative and metabolic disorders.

Figure legends.

Figure 1. Schematic summary of important MAM resident proteins involved in ER-mitochondria Ca^{2+} signaling, lipid transfer and mitochondrial morphology.
The ER-mitochondria distance varies from 10-50 nm in the sER to 50-80 nm in the rER and represents an important parameter for MAM multiple functions. To summarize, MAM play important functions in Ca^{2+} signaling (A). IP3R activation induces ER Ca^{2+} release (blue dots) and transfer from the ER to the mitochondrial matrix mediated by the interaction between the IP3R and VDAC1 through GRP75. IP3R is stabilized in MAM by the chaperone proteins SIGR1 and GRP78/BIP. Ca^{2+} transfer to the mitochondrial matrix mediated by the MCU complex stimulates mitochondrial metabolism by activating the TCA cycle through regulation of three rate-limiting enzymes: pyruvate, isocitrate, and α-ketoglutarate dehydrogenases. Furthermore, both ATP synthase and mitochondrial respiratory complex are stimulated by Ca^{2+}. Moreover, the existence of H_2O_2 nanodomains in mitochondrial cristae stimulates IP3R activity and sensitizes ER Ca^{2+} release to stimulate Ca^{2+} oscillations and mitochondrial metabolism. Excessive Ca^{2+} uptake in mitochondria causes mitochondrial Ca^{2+} overload and results in opening of the mPTP that leads to mitochondrial swelling, rupture of the OMM followed by cytochrome *c* release and apoptosis. Another important factor in Ca^{2+} signaling is the SERCA2b pump that is localized in the ER and enriched in MAM. SERCA2b is positively regulated by calnexin and negatively regulated by TMX1 in a palmitoylation-dependent manner. Moreover, VAPB and PTPIP51 form a tethering complex that is involved in Ca^{2+} transfer from ER to mitochondria. (B) MAM exert an important role in phospholipid transfer between ER and mitochondria through the expression of the MAM-enriched PSS1/2, which is involved in PS synthesis in the ER and the transfer of PS to mitochondria. PSD1 in IMM converts PS to PE. The lipid transfer ORP5/8 proteins are enriched in MAM, interact with PTPIP51 and are thought to be involved in PS transfer from ER to mitochondria. Two important enzymes, FACL4 and ACAT1/SOAT1, involved in lipid and cholesterol metabolism, respectively, are found in MAM. Another important complex containing StAR, TSPO, VDAC, ATAD3 proteins is involved in cholesterol transport from the OMM to the IMM for its conversion to pregnenolone, precursor for all steroids, mediated by the P450SCC enzyme. (C) Mitochondrial morphology is regulated by different MAM-resident proteins: MFN1/2

are involved in the mitochondrial fusion mechanism but also in ER-mitochondria tethering and regulate Ca^{2+} signaling. In the mitochondrial fission process, DRP1 phosphorylated by protein kinase A (PKA) at serine 637 is localized in the cytoplasm. When dephosphorylated by calcineurin, DRP1 is recruited to the OMM, interacts with the OMM proteins MFF and FIS1 and forms oligomers that mediate mitochondrial constriction (for further details see the main text). CE, cholesterol esters; FFA, free fatty acids; FA-CoA, fatty acids-coenzyme A; mPTP, mitochondrial permeability transition pore; PA, phosphatidic acid; PE, phosphatidylethanolamine; PS, phosphatidylserine.

Figure 2. Regulation of ER-mitochondria interaction, Ca^{2+} signaling, lipid transfer and autophagy by oncogenes, tumor suppressors, chemotherapeutic drugs and new drugs currently tested in clinical trials.

Several oncogene and tumor suppressors are found enriched in the MAM compartment and regulate important cellular pathways. Activation of Akt/mTOR pathway induces prosurvival pathways by inhibiting Ca^{2+} transfer from ER to mitochondria mediated by the phosphorylation of IP3R by Akt. mTORC2 is enriched in MAM after stimulation by growth factors where it phosphorylates Akt. Akt also regulates mitochondrial metabolism by HK2 phosphorylation and interaction with VDAC1. The Akt negative regulators PTEN and PML are enriched in MAM. The tumor suppressor PTEN modulates Ca^{2+} transfer from ER to mitochondria via its phosphatase activity on IP3R. The chemotherapeutic drug cisplatin induces apoptosis by increasing IP3R-induced ER–mitochondrial Ca^{2+} transfer. However, the antiapoptotic Bcl-2 protein inhibits cisplatin-induced apoptosis. ABT-737, a Bcl-2-inhibiting BH3 mimetic drug, restored sensitivity of cancer cells to cisplatin. Another molecule, BIRD2, acts by disrupting interaction between Bcl-2 and IP3R and induces Ca^{2+} release and apoptosis.

PTEN antagonizes FXBL2-mediated IP3R degradation by ubiquitinylation whereas the tumor suppressor BAP1 is a deubiquitylating enzyme that stabilizes IP3R and promotes Ca^{2+} transfer from ER to mitochondria. The FXBL2 inhibitor GGti-241 restores apoptosis in PTEN deregulated tumors by inhibiting IP3R3 degradation. PML is enriched in MAM and forms a complex with PP2A, Akt and IP3R and induces apoptosis by increasing Ca^{2+} transfer from ER to mitochondria. In response to stress, the tumor suppressor p53 is enriched in MAM where it directly interacts with SERCA

and increases its activity. Adriamycin renders ER–mitochondrial Ca^{2+} transfer more efficient and sensitizes cells to apoptosis by enriching p53 in MAM and increasing SERCA-mediated ER Ca^{2+} storage. G-202 (Mipsagargin) inhibits SERCAs, inducing a rise of intracellular Ca^{2+} levels and cell death. Resveratrol is an inhibitor of the ATP synthase and affects SERCA pump activity creating a high local Ca^{2+} concentration, which together with an increase of ER–mitochondrial tethering induces mitochondrial Ca^{2+} overload and apoptosis. Recently, the activated STAT3 oncogene was found in MAM where it induces IP3R degradation and reduces ER-mitochondria Ca^{2+} transfer. MAM play an important role in autophagy and represent important platforms in autophagosome formation. After starvation, the class III autophagy-specific PI3K complex involved in autophagosome formation, formed by ATG14L, Beclin 1, PI3K vacuolar protein sorting 34 (VPS34) and VPS15, is recruited to the MAM. Moreover, MAM integrity involving MFN2 and PACS2 is essential for autophagosome formation. PML and p53 MAM localization exert an important repressive role of autophagy by inhibiting autophagosome formation. Different roles of the Beclin 1/Bcl-2/ IP3R complex on autophagy induction are discussed in more details in the text.

Regulation of ER-mitochondria distance appears important in the regulation of cell survival and apoptosis. Proteins such as TpM, NOGOB and FATE1 increase ER-mitochondria distance or disrupt organelle interaction whereas VAPB and PIPTPB1 are tethering proteins and regulate ER to mitochondria Ca^{2+} transfer. Increased levels of FATE1 in adrenocortical tumors decrease mitochondrial Ca^{2+} uptake and increase resistance of cancer cells to apoptosis induced by mitotane. Mitotane is a chemotherapeutic drug that inhibits ACAT1/SOAT1, resulting in increased free cholesterol and lipid-induced ER stress. HA-15, a novel inhibitor of GRP78/BiP, and mitotane, exert together a synergic action on ER stress-induced cell death in ACC cells (for further details see the main text). EMR, emerin; FC, free cholesterol.

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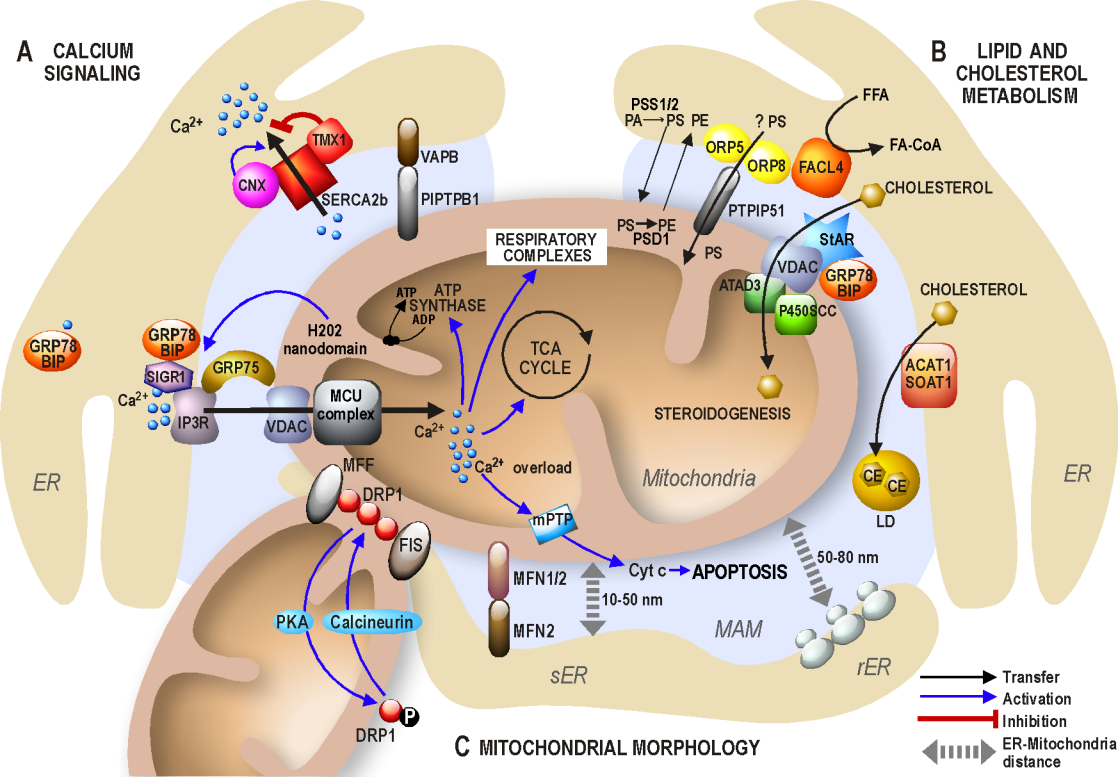
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A CALCIUM SIGNALING
B LIPID AND CHOLESTEROL METABOLISM
C MITOCHONDRIAL MORPHOLOGY
Ca2+
TMX1
CNX
SERCA2b
VAPB
PIPTPB1
PSS1/2
PA → PS
PS → PE
PSD1
? PS
ORP5
ORP8
PTPIP51
FFA
FACL4
FA-CoA
CHOLESTEROL
StAR
VDAC
GRP78 BIP
ATAD3
P450SCC
CHOLESTEROL
ACAT1 SOAT1
CE
LD
STEROIDOGENESIS
RESPIRATORY COMPLEXES
ATP SYNTHASE
ATP
ADP
TCA CYCLE
H202 nanodomain
GRP78 BIP
GRP75
SIGR1
IP3R
MCU complex
VDAC
Ca2+ overload
Mitochondria
ER
MFF
DRP1
FIS
mPTP
Cyt c → APOPTOSIS
MFN1/2
MFN2
10-50 nm
50-80 nm
MAM
sER
rER
PKA
Calcineurin
DRP1
P
Transfer
Activation
Inhibition
ER-Mitochondria distance

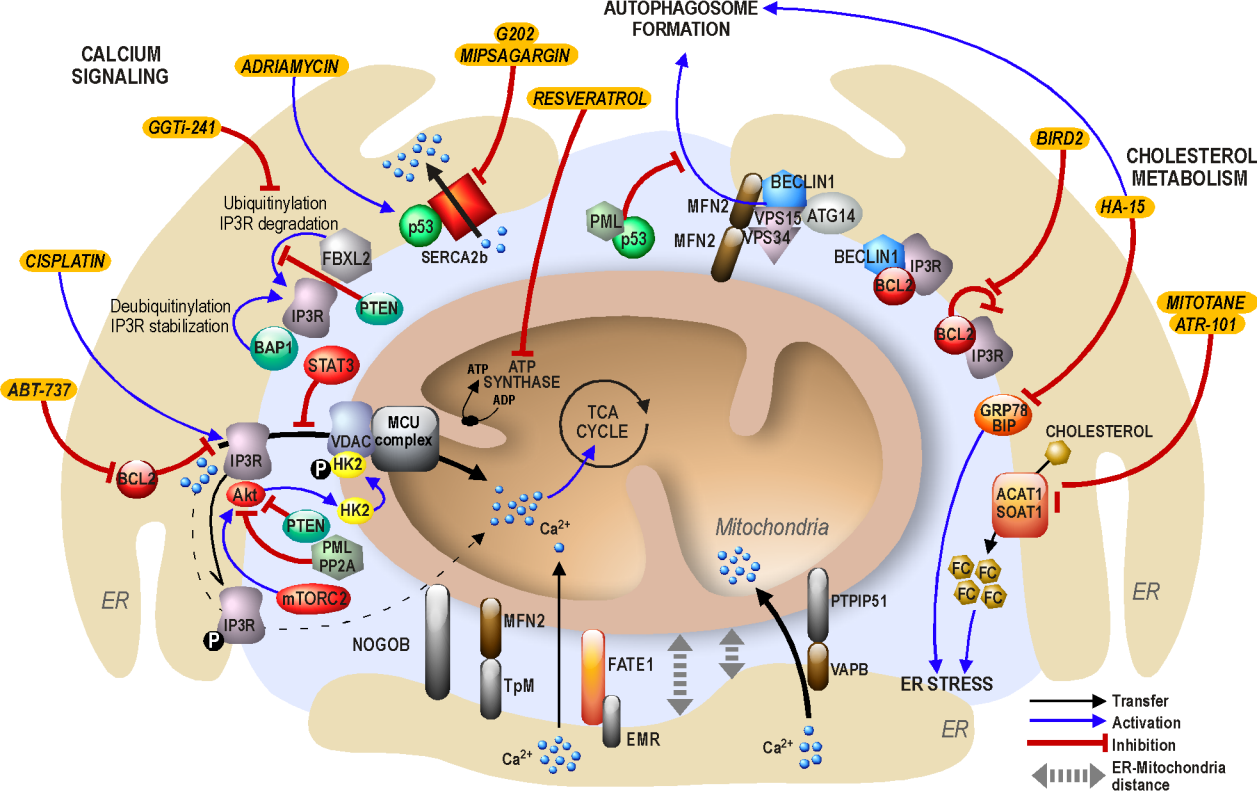

CALCIUM SIGNALING
ADRIAMYCIN
G202
MIPSAGARGIN
RESVERATROL
GGTi-241
Ubiquitinylation
IP3R degradation
FBXL2
p53
SERCA2b
AUTOPHAGOSOME FORMATION
BECLIN1
MFN2
VPS15
ATG14
VPS34
PML
p53
BIRD2
CHOLESTEROL METABOLISM
HA-15
CISPLATIN
Deubiquitinylation
IP3R stabilization
IP3R
PTEN
BAP1
BECLIN1
IP3R
BCL2
BCL2
IP3R
MITOTANE
ATR-101
STAT3
ATP
ATP SYNTHASE
ADP
TCA CYCLE
ABT-737
BCL2
IP3R
VDAC
MCU complex
HK2
GRP78 BIP
CHOLESTEROL
Akt
HK2
PTEN
PML
PP2A
Ca2+
Mitochondria
ACAT1 SOAT1
FC
FC
FC
FC
ER
mTORC2
IP3R
NOGOB
MFN2
TpM
FATE1
EMR
PTPIP51
VAPB
ER STRESS
ER
Ca2+
Ca2+
Transfer
Activation
Inhibition
ER-Mitochondria distance

Table 1. Multifaceted role of MAM: summary of the most important functional roles of some MAM-resident proteins mentioned in this review.

Principal functions	MAM resident proteins	Localization	Relevant functions in MAM	References
Ca2+ signaling	IP3R	ER, MAM	Major actor in ER-Ca2+ release to mitochondria; interacts with VDAC via GRP75	[10]
	TMX1	ER, MAM	Its palmitoyled form interacts with SERCA and inhibits its activity	[17]
	Calnexin	ER, MAM	Ca2+ binding protein that regulates SERCA activity	[17]
	SERCA	ER, MAM	Important pump involved in Ca2+ transport into the ER through an active process that requires ATP	[118]
	SIGMAR1	ER, MAM	Chaperone protein interacting with IP3R; important roles in Ca2+ transfer to mitochondria and MAM stability	[24]
	GRP75	ER, MAM, Mitochondria, cytoplasm	Chaperone protein linking IP3R and VDAC in MAM	117]
	VDAC	Mitochondria, MAM	Main Ca2+ channel localized in OMM involved in Ca2+ transport to mitochondria	[117]
Lipid Metabolism	ACAT1/SOAT1	ER, MAM	Role in cholesterol metabolism	[7]
	FACL4	ER, MAM	Role in fatty acid metabolism	[27]
	ORP5/8	ER, MAM	Role in lipid-binding and transfer	[34]
	PSS1/2	ER, MAM	Role in PS synthesis and trafficking	[7]
	StAR/ATAD3	Mitochondria, MAM	Role in cholesterol transport to mitochondria and steroidogenesis	[30, 32]
Mitochondrial Moprhology	MFN2	ER, MAM, Mitochondria	Regulator of ER–mitochondria tethering and mitochondrial fusion	[25]
ER-Mitochondria tethering	DRP1	Mitochondria, MAM, cytoplasm	Role in mitochondrial fission; interacts with MFF	[39]
	TpM	ER, MAM	Regulation of ER–mitochondria tethering by interacting with MFN2	[78]
	FATE1	ER,MAM, Mitochondria	Regulation of ER–mitochondria tethering and Ca2+ transfer from ER to mitochondria; involved in drug resistance	[87]
	NOGOB	ER, MAM	Role in ER tubulation and ER-mitochondria tethering	[80, 81]
	PTPIP51	Mitochondria, MAM	Role in Ca2+ transfer from ER to mitochondria, in lipid transfer; interacts with VAPB	[35]
	VAPB	ER, MAM	Interacts with PTPIP51	[35]
	MIRO1/2	Mitochondria, MAM	Mitochondrial GTPase involved in mitochondrial motility	[38]
Oncogenes and Tumor suppressors	Akt	PM, MAM, Mitochondria, ER	Regulation of Ca2+ signaling through phosphorylation of IP3R3; interacts with mTORC2	[51, 52]
	Bcl-2	ER, MAM, Mitochondria	Anti-apoptotic protein regulating many cellular functions	[48]
	mTORC2	ER, MAM, Mitochondria, cytoplasm	Control of MAM integrity and Ca2+ signaling via Akt regulation of PACS2, IP3R, and HK2	[58]
	STAT3	Nucleus, mitochondria, ER, MAM	Role in ER-mitochondria Ca2+ transfer by interacting with IP3R3 and facilitating its degradation	[57]
	p53	Nucleus, ER, MAM	Regulates SERCA activity and modulates ER–mitochondria Ca2+ transfer and Ca2+-induced apoptosis	[59]
	PML	Nucleus, ER, MAM	Role in regulation of IP3R Ca2+ signaling and mitochondrial Ca2+ load by interacting with IP3R, AKT, PP2A	[50]
	PTEN	Nucleus, mitochondria, ER, MAM, PM	Regulates Ca2+ release from the ER and counteracts Akt-mediated effect on IP3R	[53]
Autophagy	ATG5/ATG14	ER, MAM	Essential pro-autophagic proteins that induce autophagosome formation from MAM under starvation; interact with Beclin 1	[68]
	BECLIN 1	ER, MAM, cytoplasm	Essential pro-autophagic protein that induces autophagosome formation from MAM and interacts with Bcl-2 and IP3R	[68]